

Three cryptic species in *Asecodes* (Förster) (Hymenoptera, Eulophidae) parasitizing larvae of *Galerucella* spp. (Coleoptera, Chrysomelidae), including a new species

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Abstract

Three morphologically very similar species of *Asecodes* Förster (Hymenoptera: Eulophidae) are reviewed. *Asecodes parviclava* (Thomson) is removed from synonymy under *A. lucens* **stat. rev.**, and differentiated from *A. lucens* (Nees) and *A. lineophagum* **sp. n.** All three species develop as gregarious endoparasitoids in larvae of *Galerucella* spp. (Coleoptera: Chrysomelidae), but each species has its own unique host range. *Asecodes lineophagum* attacks only *Galerucella lineola* (Fabr.) and *A. lucens* only *G. sagittariae* (Gyllenhal), whereas *A. parviclava* parasitizes *G. tenella* (L.), *G. californiensis* (L.) and *G. pusilla* (Duftschmid). The *Asecodes* species are similar but display small though distinct morphological differences, and are distinguished also through molecular differences. The genetic distance in mitochondrial CO1 ranged from 2.3% to 7.3% between the species. Five names, one valid and four synonyms, were available for this group of species, but none of them was linked to a primary type. To promote stability of nomenclature, primary types are designated for all five names, neotypes for *Eulophus lucens* Nees, *Entedon mento* Walker and *Derostenus parviclava* Thomson, and lectotypes for *Entedon chthonia* Walker and *Entedon metagenes* Walker. *Entedon mento*, *E. chthonia* and *E. metagenes* remain synonymized under *A. lucens*.

Keywords

CO1, koinobiont endoparasitoids, host specificity, neotype designation, lectotype designation

Introduction

Species of *Asecodes* Förster are described in the literature as parasitoids of chrysomelid beetles (Askew and Viggiani 1978, Kamijo 1986, Schauff 1991). *Asecodes lucens* (Nees) (as *A. mento* (Walker)), was reared from *Galerucella nymphaeae* (L.) (Hippa and Koponen 1984) and *Lochmaea suturalis* (Thomson) (Golightly 1962) (Coleoptera: Chrysomelidae). In an unpublished study in Sweden the junior author and associates also reared *A. lucens* from five additional species of *Galerucella*, *G. californiensis* (L.), *G. lineola* (Fabr.), *G. pusilla* (Duftschmid), *G. sagittariae* (Gyllenhal) and *G. tenella* (L.). However, parasitism rates differed considerably in different *Galerucella* species collected from the same locality. These patterns caused us to wonder whether a single species, *A. lucens*, was using multiple hosts or if there were populational differences in host range. To examine the possibility that population differentiation occurs within *A. lucens* based on host use we sequenced both mitochondrial and nuclear genes (Hambäck et al. unpubl data). This analysis indicated the occurrence of multiple species.

In this paper, we identify morphological traits that separate three species of *Asecodes*, corresponding also to molecular and biological data. We compared the morphotypes with previous descriptions and found that two correspond to described species, *A. lucens* (Nees) and *A. parviclava* (Thomson), whereas the third was not described. The new species is described under the name *A. lineophagum*.

Material and methods

The colour photos were made with a Nikon SMZ 1000 stereomicroscope and a Nikon DS-5M camera. To eliminate reflections from the metallic and shiny body, a dome-light manufactured as described by Kerr et al. (2008), was used as the light source for photography. Photos were taken at different focus levels and Helicon Focus Pro version 4.75 was used to merge them into a single image. The photography of wing interference patterns is described in detail by Shevtsova and Hansson (2011). The SEM photos were made from uncoated specimens on their original cardboard mounts. These were taken in low vacuum mode on a JEOL® JSM 5600LV SEM microscope.

Morphological terms follow Gibson (1997). For illustrations of the morphological terms see www.neotropicaeulophidae.com.

The genetic distances were estimated from pairwise comparisons of 784 base pairs corresponding to the three prime end of CO1 (as used in Hambäck et al., unpublished). In the analysis, 7 individuals of *A. lucens*, 23 individuals of *A. parviclava* and 3 individuals of *A. lineophagum* were used. The analysis was done in PAUP* ver. 4.0.a125 (Swofford 2002), and genetic distances were calculated under the Kimura-2-parameter

model (K2P) with pairwise deletion of missing data. Additional mitochondrial and nuclear genes data were used for reconstructing the phylogeny (Hambäck et al., unpublished), but were not included in the estimate of genetic distances due to a less extensive data set than for CO1.

Museum acronyms

BMNH	the Natural History Museum, London, United Kingdom (N. Dale-Skey Papilloud)
CH	private collection of Christer Hansson
GNM	the Natural History Museum, Gothenburg, Sweden (C.G. Jonsson)
LUZM	Lund University Zoology Museum, Sweden (R. Danielsson)
NHRS	the Natural History Museum, Stockholm, Sweden (H. Vårdal)
ZSM	Zoologische Staatssammlung, München, Germany (S. Schmidt)

Designation of primary types

Five names were available for this complex of species prior to this investigation, with one name, *A. lucens*, considered as valid and four other names as synonyms under the latter name. However, none of the names were fixed to a primary type and three of the names lacked type material altogether. For nomenclatural stability names must be fixed to a type specimen, and we therefore designate primary types, neotypes or lectotypes, for all five available names.

Neotypes

Eulophus lucens was described by Nees from a single female caught on *Robinia pseudoacacia* in Sickershausen [Bavaria, Germany] June 22, 1812, which was placed in his own collection. Specimens in the collection of Nees no longer exist apart from specimens sent to Westwood, now in the Oxford University Museum of Natural History (Graham 1988). No material of *E. lucens* exists in the Oxford collection (Graham 1988) and it is thus safe to assume that the holotype of *E. lucens* was destroyed. A female from Hungary, Vas County, Szakonyfalu, collected 23.vi.1960, agrees well with the description of *E. lucens* and is here designated as neotype. The locality in Hungary is the closest to the type locality where it has been possible to find material of this species.

Entedon mento was described by Walker from an unspecified number of males from near London and from Belfast. There is no material that agrees with the original description, either in the general collection or in the type collection of the Natural History Museum, London, where the material Walker based his descriptions is kept. In the type collection, the box supposed to contain some type material of *E. mento* is emp-

ty, but with a note “mento??”. Thus, it appears the material on which Walker based his description of *E. mento* is now lost. A female from England, Middlesex, Southgate, collected 6.vi.1972, fits the description of *E. mento* and is here designated as neotype for *Entedon mento*. The species was allegedly described from males, but males and females of this species are very similar. Further, Walker frequently misidentified the sex (Graham 1963), and a female is selected for neotype to make this species comparable to the other species of this complex, all of which are represented by females.

Derostenus parviclava was described by Thomson from an unspecified number of females he collected on Öland, an island in the Baltic Sea, and by G.F. Möller in Holmeja, a locality in Skåne, the southernmost province in Sweden. The collection of C.G. Thomson is in the Lund museum, and the collection of G.F. Möller is in the Natural History Museum in Gothenburg, both in Sweden. However, neither collection has any specimens from the type localities of *D. parviclava*. There is a female under the name *D. parviclava* in the G.F. Möller collection from Bökeberg (labeled “Bök”), which is a locality very close to Holmeja, one of the type localities. Because Thomson was very specific concerning localities from the province Skåne, where he lived and worked, it seems unlikely that he interchanged “Holmeja” with “Bökeberg”. However, the female from Bökeberg agrees well with the original description of *D. parviclava* and it is from a locality very close to one of the original type localities. This specimen fulfills the criteria for a neotype for *Derostenus parviclava* and is designated as such here.

Lectotypes

The descriptions of *Entedon chthonia* and *E. metagenes* do not have information on the number of specimens used, and neither has been fixed to a primary type. The type collection of the Natural History Museum, London, has a specimen each of *E. chthonia* (type no. 5.2603) and *E. metagenes* (type no. 5.2604). These specimens fit the original descriptions and are here designated as lectotypes.

Biology of the parasitoids

The *Asecodes* species included here are gregarious koinobiont endoparasitoids of beetle larvae (Stenberg and Hambäck 2010). Females lay their eggs in the early larval stage and successful parasitoid development leads to a mummification of the host larva. The host larval mummies are black and morphologically resemble larvae, whereas unparasitized larvae form soft yellow pupae typical of chrysomelid beetles (photos in Hambäck 2004). After successful development the parasitoid larvae pupate inside the mummified host larva. The number of parasitoid pupae in the mummies is highly variable, from 1–14 within one mummy. The emergence of adult parasitoids typically occurs in intervals, which probably reflects separate egg-laying events and thus indicates superparasitism as a common trait. The number of parasitoids in each host affects both the sex ratio

and the adult body size of emerging offspring. Single emerging parasitoids are invariably females, but at high parasitoid densities the sex ratio is male biased (up to 80%) (Stenberg and Hambäck 2010). It also seems that density dependence in sex ratio and adult body size are correlated with host species, as indicated by a comparison of parasitoids emerging from *Galerucella tenella* and *G. californiensis* (Stenberg and Hambäck 2010).

Parasitism rates may at times be very high, close to 100%, but may at other times be very low. In Sweden, where this study was performed, there seems to be a latitudinal shift in parasitism rates, at least for some hosts. The parasitism rates for *G. californiensis* and *G. tenella* in northern localities, close to Umeå, are typically very high, between 50% and 100%, but less than 10% in more southern localities. The genus contains both strictly monophagous species and oligophagous species and the different *Galerucella* species often occur in the same localities, but on different wetland plants. There are often large differences in the parasitism rates between *Galerucella* species within the same locality. For instance, parasitism rates may be very high on *G. lineola* and very low on other *Galerucella* species in one locality, whereas parasitism rates are high in another species in another locality. The different parasitism rates are not likely to be due to phenological differences or spatial distributions within localities because host plants colonised by different larval species may occur on neighboring plant individuals.

Genetic analysis

Genetic distances of *A. lucens* vs *A. lineophagum* calculated from mitochondrial gene data varied between 4.8–6.0 % (mean = 5.3%), *A. lucens* and *A. parviclava* 5.3–7.3 % (mean = 6.4%) and *A. lineophagum* vs *A. parviclava* 2.3–3.8% (mean = 3.0%). Variation was estimated as 2.6% for *A. parviclava*, whereas no variation was found within *A. lucens* and *A. lineophagum* for the sampled individuals.

Identification

For identification of the species treated here the following additions can be made to the latest key to European species of *Asecodes* in Askew and Viggiani (1978).

Couplet 9, replace “mento” with “11”, and include the following:

- | | |
|----|--|
| 11 | Forewing speculum open (Fig. 2); propodeal callus with 3–5 setae (Fig. 22).
..... <i>A. lineophagum</i> sp. n. |
| – | Forewing speculum closed posteriorly by costal setal line (Fig. 6); propodeal callus with 2 setae (Fig. 23)..... 12 |
| 12 | Forewing bare just behind marginal vein, and relatively sparsely setose (Figs 4, 9, see also Fig. 2) <i>A. parviclava</i> (Thomson) |
| – | Forewing setose just behind marginal vein setose, and relatively densely setose (Figs 6, 8) <i>A. lucens</i> (Nees) |

Species treatments

Asecodes lineophagum sp. n.

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http://species-id.net/wiki/Asecodes_lineophagum

Figures 2, 3, 10, 13, 16, 17, 22, 26, 27

Diagnosis. Forewing (Fig. 2) with speculum open posteriorly (i.e. setal line absent), bare just behind marginal vein and otherwise relatively sparsely setose; propodeal callus with 3–5 setae (Fig. 22).

Description. FEMALE. Length 1.0–1.8 mm.

Antenna dark brown (Fig. 10). Frons below frontal suture metallic purple with upper-lateral corners close to eyes and frontal suture golden-green (Fig. 26), above suture golden green. Vertex metallic purple inside ocellar triangle, golden-green outside triangle. Mesoscutum black with metallic purple tinges (Fig. 16). Scutellum metallic bluish-green (Fig. 16). Axillae black with metallic purple tinges (Fig. 16). Dorsellum metallic bluish-green (Fig. 16). Propodeum metallic bluish-green (Fig. 16). Coxae, femora and tibiae dark brown to black, and shiny (as in Fig. 1); fore tarsus dark brown, mid and hind tarsi with tarsomeres 1–3 yellowish-white, tarsomere 4 dark brown. Forewing hyaline (Fig. 2), wing interference pattern as in Fig. 3. Petiole dark brown to black. Gaster with 1st tergite metallic bluish-green, remaining tergites dark brown to black with metallic purple tinges.

Antenna as in Fig. 10. Frons below frontal suture with weak reticulation, above suture smooth; antennal scrobes join on frontal suture. Vertex with very weak reticulation inside ocellar triangle, smooth outside triangle.

Mesoscutum with weak reticulation (Fig. 17). Scutellum with very weak reticulation in anterior 2/3 (Fig. 17), posterior 1/3 smooth. Axillae with weak reticulation (Fig. 17). Dorsellum slightly convex and smooth (Fig. 17). Propodeum with a wide groove along anterior margin (Fig. 17), with weak reticulation; propodeal callus with 3–5 setae. Forewing (Fig. 2) bare just behind marginal vein, speculum open, setation relatively sparse, and with 5–9 admarginal setae.

Petiole as a short, transverse, narrow stripe. Gaster circular.

Ratios. Height of eye/malar space/width of mouth = 2.6/1.0/1.8; shortest distance between posterior ocelli/posterior ocellus and eye/posterior ocellus and occipital margin = 10.4/5.4/1.0; width of head/width of mesosoma = 1.1; length of forewing/length of marginal vein/height of forewing = 2.4/1.0/1.0; length of postmarginal vein/length of stigmal vein = 0.6; length of mesosoma/length of gaster = 1.0.

MALE. Length 0.9–1.4 mm.

Very similar to female except antenna (Fig. 13) with scape wider, flagellomeres longer and more slender, and apical two flagellomeres distinctly separated.

Ratios. Height of eye/malar space/width of mouth = 2.1/1.0/1.6; length of mesosoma/length of gaster = 1.0–1.2.

Hosts and sex ratio. All Swedish specimens were reared from *Galerucella lineola* (Coleoptera: Chrysomelidae) on *Salix* spp., mainly *S. cinerea*. The sex ratio of each clutch on average is closer to one than for *A. lucens*, and the standard deviation is distinctly higher in *A. lineophagum*. Ratio female/male ($n = 48$): $3.10 \pm 2.68 / 2.27 \pm 2.17$

Material examined. HOLOTYPE female (BMNH) labelled “SWEDEN: Uppland, Ludden, 59°46'18"N, 18°40'19"E, 21.vi.2011, ex *Galerucella lineola* on *Salix cinerea*”. PARATYPES. 45♀ 49♂ (BMNH, CH, LUZM, NHRS, ZSM): 25♀ 19♂ with same label data as holotype; 3♀ 1♂ “Sweden: Uppland, Fläktan, 59°46'54"N, 17°44'30"E, 24.vii.2011, ex *Galerucella lineola* on *Salix cinerea*.”; 3♀ 5♂ “SWEDEN: Uppland, Liljekonvaljholmen, 59°48'18"N, 17°39'51"E, 18.vi.2011, ex *Galerucella lineola* on *Salix* sp.”; 6♀ 4♂ “SWEDEN: Uppland, Mörtsjön, 59°38'39" N 18°09'58" E, 5.vii.2011, ex *Galerucella lineola* on *Salix cinerea*”; 1♂ “SWEDEN: Uppland, Sundängen, 59°33.954'N, 16°51.292'E, 25.vi.2011, ex *Galerucella lineola* on *Salix cinerea*”; 3♀ 6♂ “SWEDEN: Uppland, Haknäs, 59°43'05"N, 17°41'57"E, 25.vi.2011, ex *Galerucella lineola* on *Salix cinerea*”; 1♀ “SWEDEN: Skåne, Skäralid, 6–17.viii.1994, M. Sporrang”; 3♀ (on two pins) labelled “Småland” [which is: SWEDEN: Småland, without further information]; 1♀ 1♂ “SWEDEN: Skåne, Sövde, 5.vii.1985, C. Hansson”; 1♂ “SWEDEN: Småland, Hyltebruk, 7–14.ix.1986, J. Ardö”; 1♂ “NORWAY: Jostedalen, Gupne, 19.vii.1979, Hull University Expedition”.

Asecodes lucens (Nees)

http://species-id.net/wiki/Asecodes_lucens

Figures 6–8, 12, 15, 20, 21, 23, 24

Eulophus lucens Nees, 1834: 175. Neotype female in ZSM, designated here.

Entedon mento Walker, 1839: 28. Neotype female in BMNH, designated here. Synonymized by Graham (1993: 227).

Entedon chthonia Walker, 1839: 122. Lectotype female in BMNH, designated here. Synonymized by Graham (1993: 227).

Entedon metagenes Walker, 1848: 230. Lectotype female in BMNH, designated here. Synonymized by Graham (1993: 227).

Asecodes lucens (Nees), Graham (1993: 227).

Diagnosis. Forewing (Figs 6, 8) with speculum closed posteriorly by a setal line, setose just behind marginal vein and otherwise relatively densely setose; propodeal callus with 2 setae (Fig. 23).

Hosts and sex ratio. All Swedish specimens were reared from *Galerucella sagittariae* (Coleoptera: Chrysomelidae) on *Lysimachia thyrsiflora*, *L. vulgaris*, and *Potentilla palustris*. The sex ratio of each clutch is female biased. Ratio female/male ($n = 48$): $4.27 \pm 1.83 / 0.92 \pm 0.94$.

Material examined. TYPE MATERIAL: Neotypes of *E. lucens* (ZSM) and *E. mento* (BMNH), lectotypes of *E. chthonia* (BMNH) and *E. metagenes*

(BMNH), all types are females. ADDITIONAL MATERIAL: DENMARK: 4♀ 2♂(CH, LUZM). HUNGARY: Vas Co. 5♀ 2♂(ZSM). SWEDEN: Skåne 106♀ 13♂(swept) (BMNH, CH, LUZM); Uppland 205♀ 44♂(91♀ 25♂ from *Lysimachia*, 114♀ 19♂ from *Potentilla palustris*) (BMNH, CH, NHRS, ZSM); Öland 1♂(swept) (CH).

***Asecodes parviclava* (Thomson), stat. rev.**

Figures 1, 4, 5, 9, 11, 14, 18, 19, 25

Derostenus parviclava Thomson, 1878:272–273. Neotype female in GNM, designated here.

Asecodes parviclava (Thomson), Bouček and Askew (1968: 131).

Synonym of *Asecodes lucens* (Nees) (Graham 1993: 227).

Diagnosis. Forewing (Figs 4, 9) with speculum closed posteriorly by a setal line, bare just behind marginal vein and otherwise relatively sparsely setatose; propodeal callus with 2 setae (as in Fig. 23).

Hosts and sex ratio. The Swedish specimens were reared from *Galerucella californiensis* and *G. pusilla* (Coleoptera: Chrysomelidae) on *Lythrum salicaria*, and *G. tenella* on *Filipendula ulmaria*. The number of samples is smaller than for the other two species (n = 23, 10 from *G. californiensis*, 6 from *G. pusilla*, 7 from *G. tenella*). Ratio female/male: 2.09±1.16/0.91±0.95.

Material examined. TYPE MATERIAL: Neotype female of *D. parviclava* (GNM). ADDITIONAL MATERIAL: HUNGARY: Vas Co. 1♀ (BMNH); SWEDEN: Skåne 21♀ 16♂(CH, LUZM); Uppland 48♀ 21♂(24♀ 8♂ from *G. californiensis*, 10♀ 8♂ from *G. pusilla*, 14♀ 5♂ from *G. tenella*) (BMNH, CH, NHRS); Västergötland 14♀ 5♂(CH, LUZM).

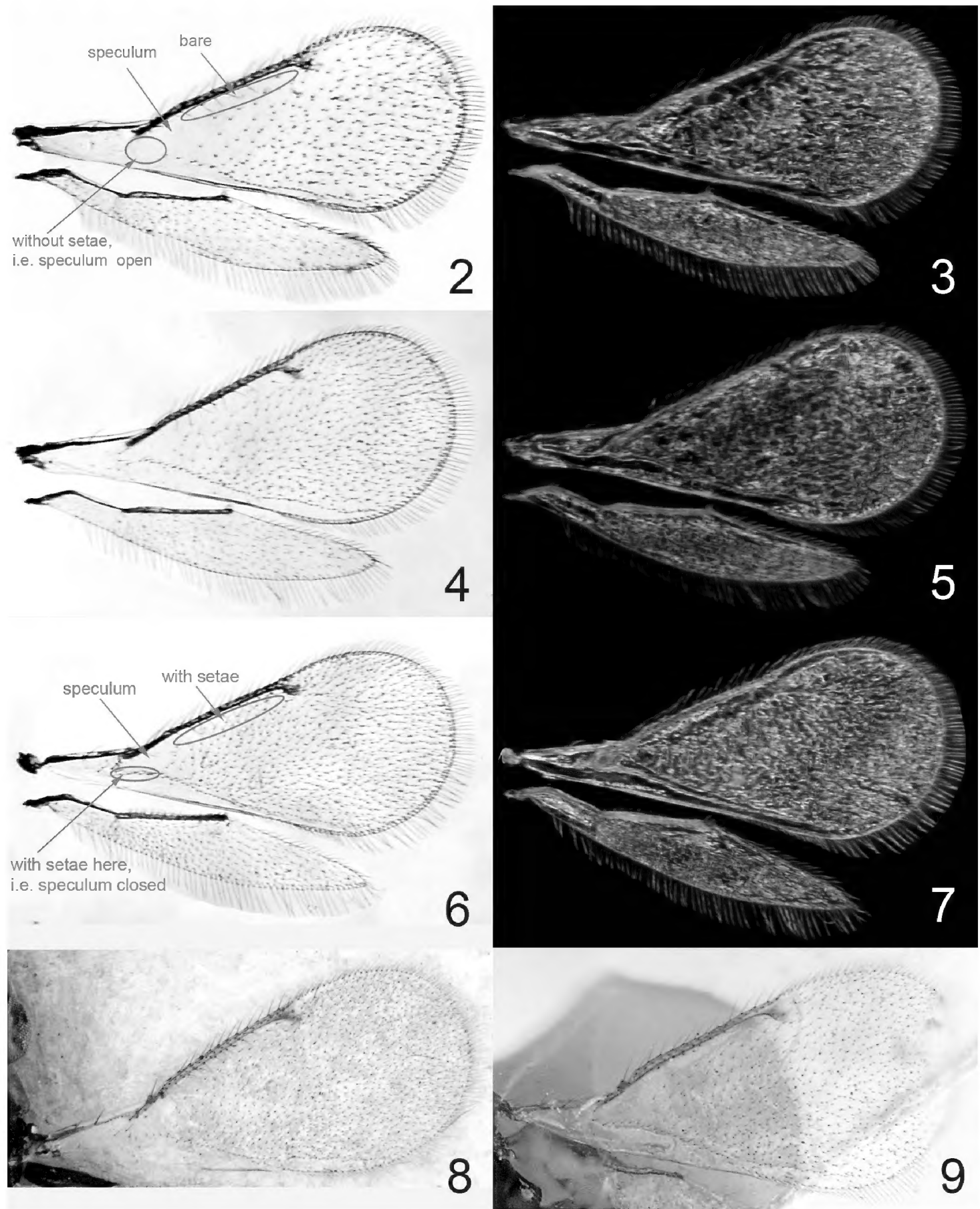
Discussion

The separation of *A. lucens* into three species based on molecular and morphological evidence is supported by biological data. *Asecodes lucens* and *A. lineophagum* were reared from only one host species, *Galerucella sagittariae* and *G. lineola*, respectively, whereas *A. parviclava* was reared from three host species, *G. tenella*, *G. californiensis* and *G. pusilla*. These observations have also been confirmed with independent observations of parasitoid behaviour in the laboratory, where females were found to attack the respective host species, but not other species (L. Fors, unpubl. data). The delimitation of three species is also supported by observations in the field, where one species of *Galerucella* larvae may be heavily parasitized and another is not attacked in the same locality. Such parasitism patterns have, however, not been observed for *G. tenella*, *G. californiensis* and *G. pusilla*. In fact, earlier studies show



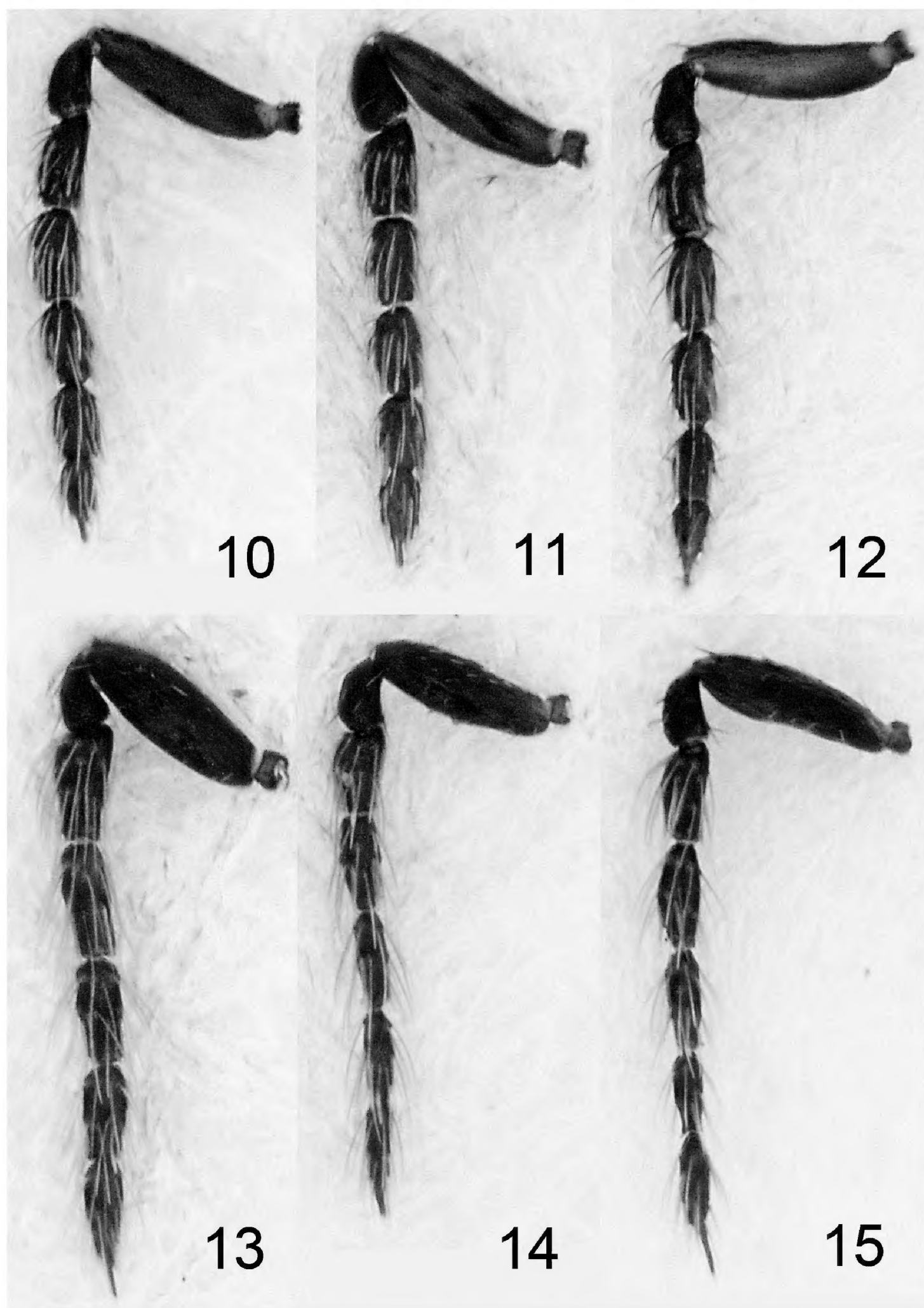
Figure 1. *Asecodes parviclava* (Thomson), female habitus (length = 1.5 mm).

strong correlations in parasitism rates between *G. tenella* and *G. californiensis* among localities (Hambäck et al. 2006). Moreover, field observations suggested that parasitism rates on *G. tenella* were consistently higher when this species was sympatric with *G. californiensis*. The reason for this pattern was not resolved (Hambäck et al. 2006), but the pattern suggest that the parasitoid population may mediate indirect interactions between its hosts, as is known for other host-parasitoid systems. The current information on the species delimitation within *Asecodes* was important to identify pairs of host species where such effects would be likely. Based on previous information, we could have expected similar indirect effects also for other species pairs but the novel information on population differentiation among parasitoid individuals suggest this not to be the case.

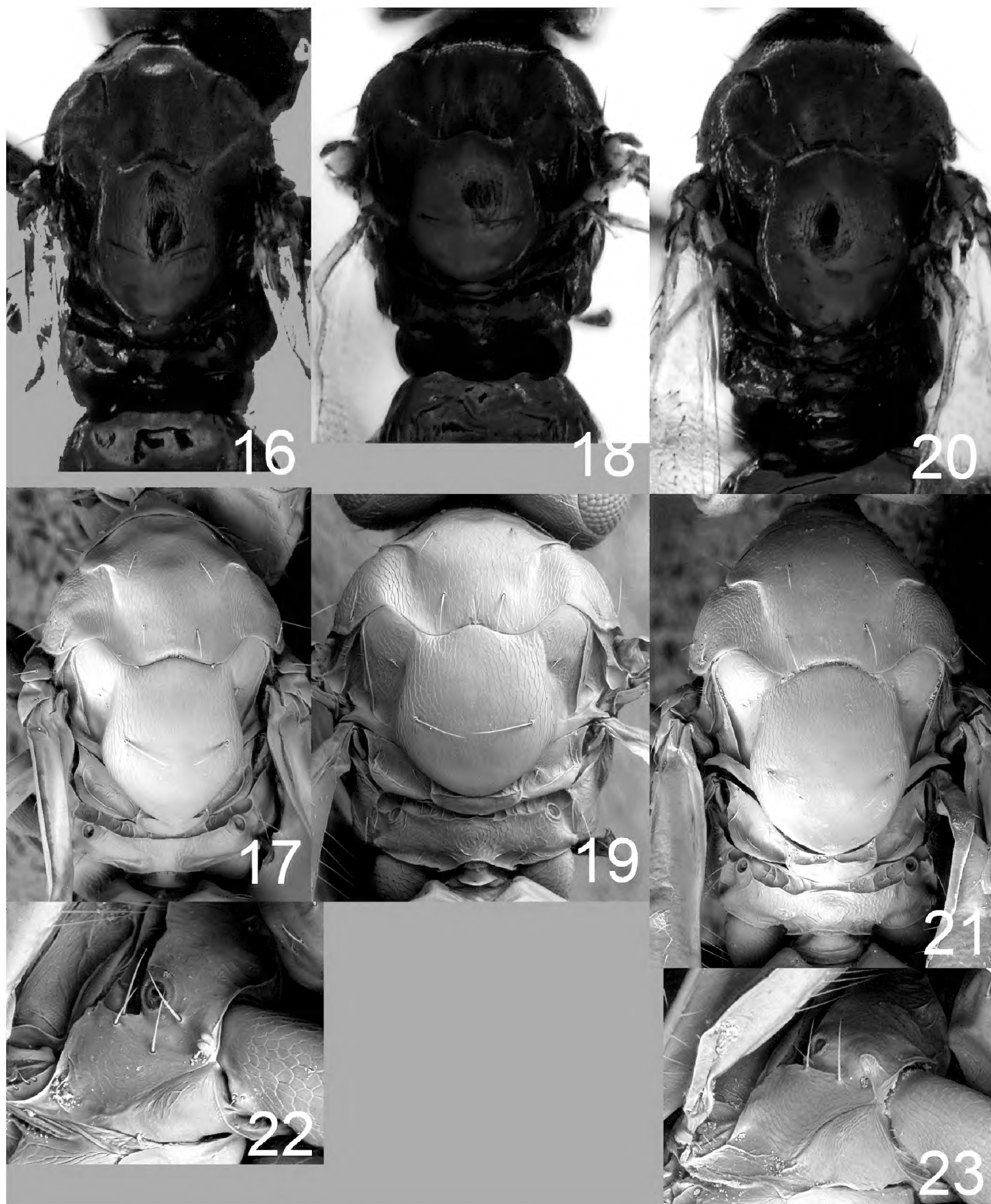


Figures 2–9. *Asecodes* spp., wings, females **2–3** *A. lineophagum* sp. n. **2** transparent wings **3** wing interference pattern **4–5** *A. parviclava* (Thomson) **4** transparent wings **5** wing interference pattern **6–7** *A. lucens* (Nees) **6** transparent wings **7** wing interference pattern **8** *A. lucens*, forewing, neotype **9** *A. parviclava*, forewing, neotype.

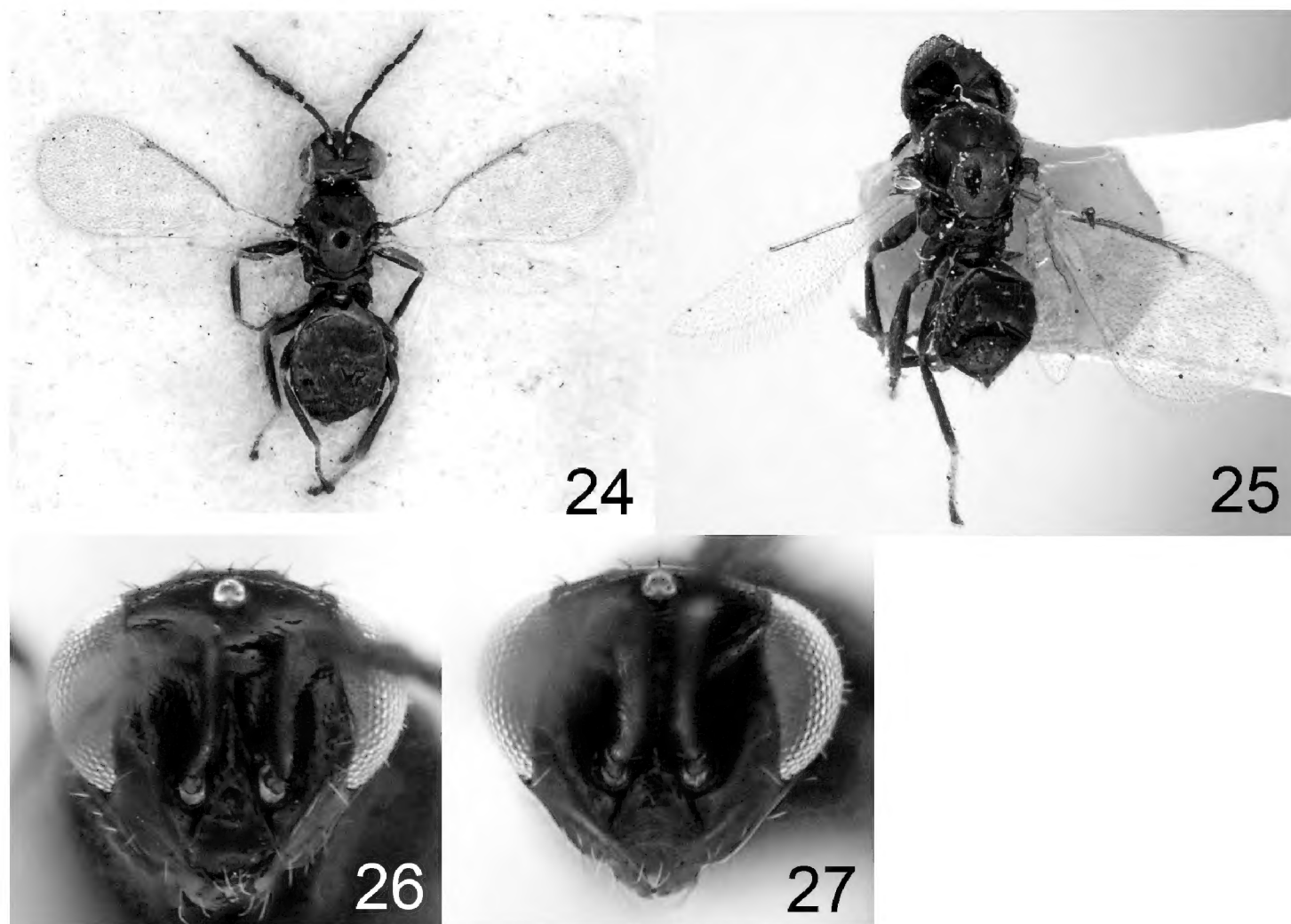
In view of our findings, the previous host records of *Galerucella nymphaeae* and *Lochmaea suturalis* for *A. lucens* need confirmation. Investigation of *Asecodes* specimens reared from these hosts might quite possibly reveal additional cryptic species in this group.



Figures 10–15. *Asecodes* spp., antennae **10–12** females **10** *A. lineophagum* sp. n. **11** *A. parviclava* (Thomson) **12** *A. lucens* (Nees) **13–15** males **13** *A. lineophagum* **14** *A. parviclava* **15** *A. lucens*.



Figures 16–23. *Ascodes* spp. **16–21** thoracic dorsum, females **16–17** *A. lineophagum* sp. n. **18–19** *A. parviclava* (Thomson) **20–21** *A. lucens* (Nees) **22–23** lateral propodeum in side view (anterior part to the left), female **22** *A. lineophagum* **23** *A. lucens*.



Figures 24–27. 24–25 *Asecodes* spp., neotypes, females **24** *A. lucens* (Nees) **25** *A. parviclava* (Thomson) **26–27** *A. lineophagum* sp. n., head in frontal view **26** female **27** male.

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